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THE USE OF ARTIFICIAL INTELLIGENCE FOR THE IDENTIFICATION OF BACTERIA

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A method has been investigated to provide more rapid and more efficient identification of bacteria using artificial intelligence, a process in which a computer can examine a variety of facts and devise a solution by comparing the facts with a data base. Incorporated into the data base are the names of 564 species of medically important bacteria with 0-90% positive or negative results of biochemical reactions for the identification of each species. In a rapid search of the data base, the computer selects the three most likely organisms with a likelihood index for each. This application of artificial intelligence eliminates tedious matching with biochemical charts; it can be used by the less skilled technician.

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THE USE OF ARTIFICIAL INTELLIGENCE FOR THE IDENTIFICATION OF BACTERIA

INTRODUCTION

In the early and mid-1960's a number of investigators working independently began a search for a way to use existing mathematical models to formulate biochemical data bases for bacterial identification (1,2,3,4). These programs are mathematical manipulations of a data base matrix utilizing some form of Bayesian probability. Bacterial identification through computer manipulation of a large data base could be performed faster and more accurately than most microbiologists could achieve with identification charts (4,5). Manufacturers of commercial kits and automated and semi-automated equipment have effectively used these procedures to identify the more commonly isolated organisms.

METHODS AND MATERIALS

In 1985 a cooperative study was undertaken by the Epidemiology Division and the Data Services Branch, Technical Services Division, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas to determine the possibility of using artificial intelligence for bacterial identification. The bacterial identification system uses conventional biochemical tests with daily readings, for periods ranging from 2 to 7 days (Fig. 1).

The protocols for testing are taken from published data of the Centers for Disease Control, American Type Culture Collection and Manual of Clinical Microbiology, 4th edition. Definitive identification of genus, species, and biotype is achieved through the comparison to charts representing large numbers of organisms which have been tested with each biochemical test, providing a percentage positive and a percentage negative reaction for each test (Fig. 2).

The data base consists of genus and species names of 564 organisms with biotypes as appropriate; all possible biochemical reactions 0-90% positive or negative; flags to alert the microbiologist to unusual or serious pathogens; recommendations for further tests; and probability percentages for closely related organisms.

At this point every test result obtained on the unknown is compared against every organism in that particular group. No expert knowledge about individual test results versus particular organisms is applied at this point. Every test has equal weight. This is a Bayesian-like procedure, but since the original population of the unknown is not known, the a priori probabilities of occurrence of particular organisms is unknown. Therefore, the numbers obtained to rank each organism are not probabilities but likelihoods. These likelihoods are ranked, thus reducing the search space to the top three most likely candidates.

The expert system has now reached the goal state, but with three candidates. Since the goal has been reached without using any rules to exclude particular organisms, the program now backward chains from those candidates to advise the microbiologist of any inconsistencies between the test results and the three candidates under consideration. Subroutines have been coded for each family group which contain expert knowledge about individual organisms.

These rules are then applied to the three candidates. Another subroutine, which is optional, can search the data base on its own and advise the human expert on situations which are highly unlikely.

After these rules are applied, the decision of the most likely candidate is left to the microbiologist (Fig. 3).

Gram's stain results and cultural characteristics dictate the specific protocol for testing. There are 15 specific biochemical protocols or lists used: List #1=Enterobacteriaceae; List #2=Vibrionaceae; List #3=Capneic gram-negative bacilli and coccobacilli; List #4=gram-negative nonfermenting bacilli; List #5=Hemophilus species; List #6=Fastidious gram-negative rods and coccobacilli; List #7=Streptococcus species; List #8=Corynebacterium species, and related gram-positive bacilli; List #9=gram-negative cocci; List #10=Anaerobes; List #11=Staphylococcus species; List #12=Listeria species and Erysipelothrix; List #13=Lactobacillus species; List #14=Bacillus species; and List #15=Unusual gram-negative bacilli such as *Simonsiella* and *Erwinia*. These lists can be shown on the computer format by requesting the identification program 5 or 11 (Fig. 4).

Artificial intelligence can now provide expert status for bacterial identification. Expert status is defined as the ability of this system to identify isolates as well as, or better than, a microbiologist using charts. The computer printout is much more rapid than visual comparison and presents numerical likelihood of identification for the three most likely organisms.

The biochemical tests performed for each test list are in the appendix. These biochemical tests are available on the computer format when requested from the program menu. The operator enters the results of each test by pressing P for positive, N for negative, and I for test not performed. The results are displayed on the screen with command capability to change erroneous entry prior to the command for data search and display of the bacterial identification. After the three most probable organisms are listed in probability order, the command (continue) can be given to ascertain the positivity index identification (Figs. 5 and 6).

EDITOR'S NOTE: For the convenience of the reader, figures have been placed at the end of the report.

SUMMARY OF CONCLUSIONS

At this time, artificial intelligence has provided a system which identifies isolates as well as, or better than, the microbiologist using extensive charts. The computer identification is much more rapid than visual comparison and identifies the three organisms having the highest confidence levels. The user is also advised of unlikely test reactions and given recommendation for further tests if required.

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BIOCHEMICAL REACTIONS			
(THIS FORM IS SUBJECT TO THE PRIVACY ACT OF 1974 - USE BLANKET PAS - DD FORM 2005)			
EL NUMBER GRAM STAIN B6-10	DATE 6 Feb 87	NAME Doe, Jane	
Gram negative cocci			
TCBS AGAR	CAMPY AGAR	BASE	Any place AFB
MACCONKEY AGAR -	BLOOD AGAR +	RESULTS	
SS AGAR -	CHOCOLATE AGAR +		<i>Brantamella catarrhalis</i>
Carbohydrate	Enteric	O/F Sp	PRB + XV (CTA)
Lactose Neg	HIA:		Spores
Dextrose Neg Gas -	25° C +		Pseudo Agar
Sucrose Neg	37° C +		Hemolysis
Maltose Neg	42° C Neg		Pigment (Blood)
Mannitol Neg	Lysine		Melibiose
Xylose Neg	Ornithine		Hippurate
Glycerol Neg	Arginine		Starch Hyd
O/F Medium (Dex)	Tyrosine Agar Neg		5% Suc Agar
Open Neg	Acetamide		5% Suc Broth
Closed Neg	Tartrate	Neg	Veg Hyphae (25@30°C)
Malonate	Acetate	Neg	Motility (wet prep)
Urea Neg	Mucate	Neg	Serotype No
Motility 25/37° C	Rhamnose		Methyl Red
Gelatin Neg	Arabinose		CDC Blood Agar
Indol R/HIB Neg	Dulcitol		CNA Suppl Agar
Nitrate/Nitrite +/ -	Inositol		KVBAP Agar
VPI/VP So Neg	Adonitol		BBE Agar
S. Citrate Neg	Salicin		Thayer-Martin Agar
Nut Agar 25/37° C +/+	Trehalose		Yersinia Agar
Oxidase +	Fructose	Neg	Anaerobic
Litmus Milk Neg	Sorbitol		CO ₂ Growth Only
Catalase +	Cellibiose		Amyl/sucrose Neg
Centrimide	Raffinose		
Aesculin	Arabitol		
TSI	6% NaCl		
Butt	10% NaCl		
Slant	M B Milk		
H2S (TSI)	ONPG	Neg	
Butt	J Tartrate		
Paper +	Phenylalanine +		
Pseudo F	KCN		
Pseudo P	DN'ase 25/37°C +		
	Lecithinase		
	Lipase		
	TSA (pigment, 25 C)		

AFSC Form 3290, MAR 88 PREVIOUS EDITION WILL BE USED

Figure 1. After an organism's growth characteristics and reactions have been evaluated, the technician records the results on AFSC Form 3290, Biochemical Reactions, for later data entry.

Gram - rods	Pseudomonas stutzeri	Xanthomonas (Pseudomonas) maltophilia			Pseudomonas diminuta	Pseudomonas putrefaciens Biotype 1			Pseudomonas putrefaciens Biotype 2
		Pseudomonas mendocina	O/F sp.	O/F sp.		O/F sp.	O/F sp.	O/F sp.	
O/F	+	100%	+	100%	-	+	97%	-	100%
RacConkey SS	+	96%	+	100%	d	98%	18	8%	+
Carbohydrate Base	-	100%	-	0%	d	60%	0%	d	50%
Lactose	-	100%	-	100%	a	90%	d	d	96%
Fructose	-	0%	-	0%	d	64%	-	-	0%
Sucrose	-	100%	-	0%	A	100%	-	0%	-
Maltose	-	100%	-	0%	-	0%	A	A	92%
Fructose	d	89%	-	0%	-	0%	-	0%	-
Xylose	-	93%	A	100%	d	36%	-	0%	0%
Glycerol	-	100%	A	92%	-	1%	-	0%	0%
Fructose	d	91%	d	100%	d	75%	-	0%	0%
10% Dex/10% Lact	A/d	A/-	A/-	d/-	d/-	d/-	d/-	d/-	d/-
Urea	d	51%	d	33%	d	28%	d	13%	d
Motility 25/35°C	+/+	100%	+/+	92%	+/+	97%	+/+	100%	+/+
Pept. Hydrolysis	-	1%	-	0%	+	93%	d	68%	+
Indol	-	0%	-	0%	-	0%	-	0%	0%
Nitrate/Gas from NO ₃	+/+100/100%	+/+100/100%	d/d	85/85%	d/-	39/0%	-/-	3/0%	+/+
Nitrite/Gas from NO ₂	+/+	96/96%	-	0%	0/0%	-/-	0/0%	-/-	100/0%
VP	-	0%	-	0%	d	0%	-	0%	+
Simmons citrate	+	96%	+	100%	d	46%	-	0%	-
Nutrient Agar	+	100%	+	100%	+	100%	+	100%	-
Oxidase	+	100%	+	100%	d	32%	+	100%	+
Litmus Milk	K	89%	K	84%	Rep	9b%	V	+	8%
Catalase	+	100%	+	100%	+	98%	+	100%	-
Pseudos:	d	17%	+	100%	-	6%	-	0%	0%
Aesculin	-	0%	-	0%	d	39%	-	5%	0%
1/2S Burt/Paper	-/d	0/36%	-/+	0/100%	-/+	0/95%	-/d	0/34%	+/+ 100/100%
Tomato juice	+	84%	+	100%	+	98%	+	100%	d 16%
Growth at 25/35/42°C	+/-/d	+/-/+	+/-/d	+/-/d	+/-/d	+/-/d	+/-/d	+/-/d	+/-/d
Lysine decarbox.	-	0%	-	0%	+	94%	-	0%	-
Ornithine decarbox.	-	0%	-	0%	-	0%	-	0%	+
Arginine Dihydro.	d	68%	+	100%	-	0%	-	0%	0%
Tyrosine Agar	-	17/0%	d/-	14/0%	-/-	0/0%	-/-	0/0%	Brown(-) 0/0%
Acetamide/Tartrate	+/-	100/50%	+/-	100/85%	+/-	100/0%	+/-	100/0%	-/- 0/0%
Acetate/Fluicate	-	3%	-	0%	+	97%	-	0%	+/- 100/0%
IN'ase	d	22%	-	0%	d	75%	-	0%	+ 100%
Pseudomonas Agar	-	-	-	-	-	-	d	d	d

^aRequires minimum of 0.5% NaCl for growth

Figure 2. This Centers for Disease Control (CDC) chart is used for manual identification of bacteria.

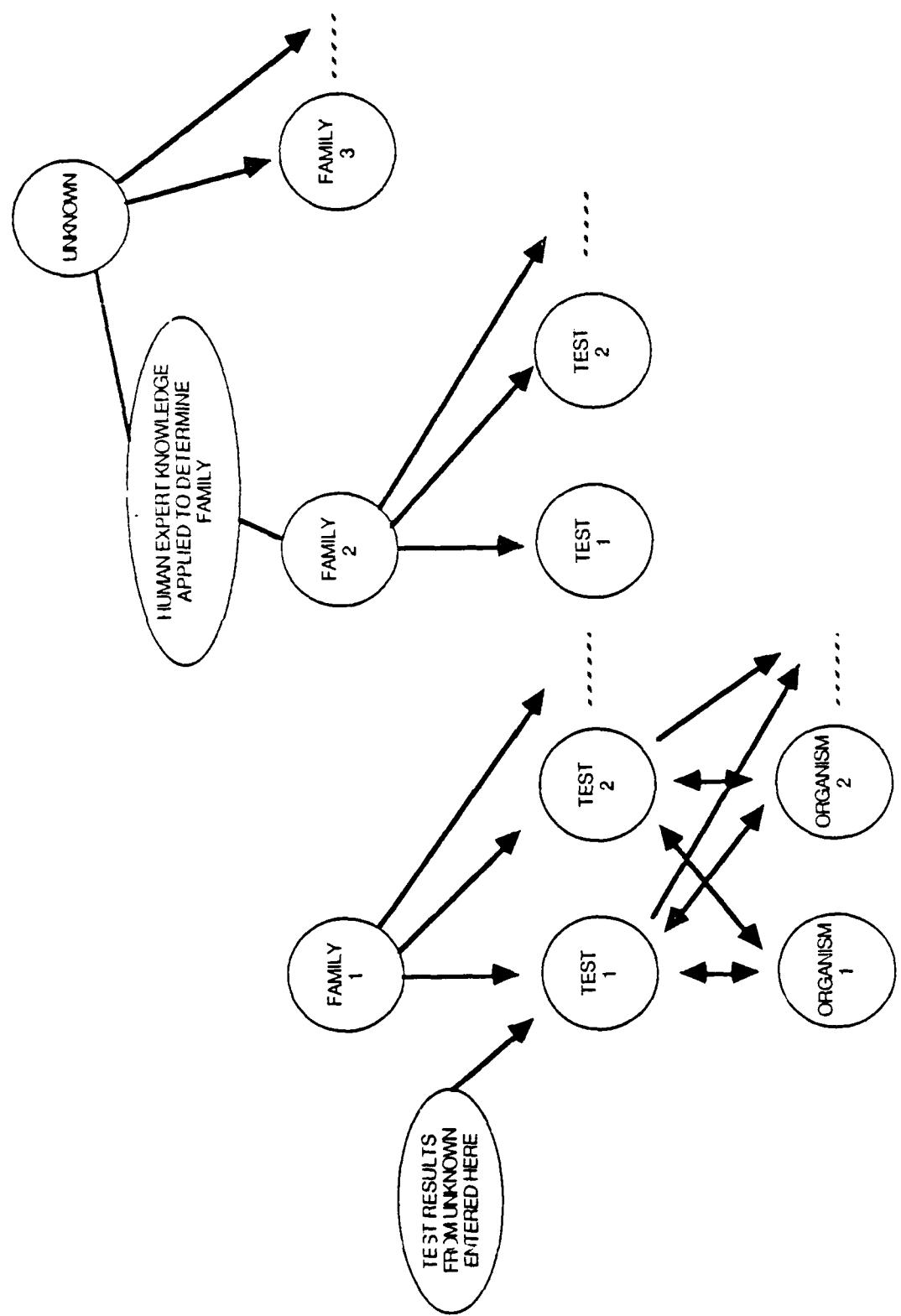


Figure 3. The expert system is a classification system in which knowledge about the unknown is forward chained to the goal state. The goal state is a particular known organism which most closely matches the knowledge about the unknown organism.

MENU FOR GROUP SELECTION

- 1 Enteric
- 2 Vibrionaceae
- 3 Gram neg capneic rods
- 4 Non ferm gram neg
- 5 Hemophilus
- 6 Fastidious gram neg
- 7 Gram pos cocci
- 8 Gram pos rods (non-sporeformers)
- 9 Gram neg cocci
- 10 Anaerobic
- 11 Staphylococcus
- 12 Listeria - Erysipelothrix
- 13 Lactobacillus
- 14 Large gram pos rods (sporeformer)
- 15 Unusual gram neg rods

Please enter group number:

Figure 4. After evaluating the organism's cultural characteristics, the technician selects one or more specific protocols (lists) from the program menu.

EKLM TEST RESULTS

1 OXIDATIVE	N	2 FERMENTATIVE	P	3 LACTOSE	N
4 DEXTROSE	P	5 SUCROSE	N	6 MALTOSE	P
7 MANNITOL	N	8 XYLLOSE	N	9 GLYCEROL	P
10 FRUCTOSE	P	11 UREA	N	12 MOTILITY	N
13 GELATIN	P	14 INDOL	N	15 NITRATE	P
16 S. CITRATE	N	17 NUTRIENT AGAR	P	18 BROTH+6% NaCl	P
19 OXIDASE	N	20 LITMUS MILK	P	21 CATALASE	P
22 AESCULIN	N	23 H2S, PAPER	P	24 GROWTH, 25 C	P
25 GROWTH, 35 C	P	26 GRCWTH, 42 C	N	27 DNASE	P
28 PIGMENT	N	29 TINSDALE, HALO	P	30 TOXIGENIC	N
31 VP	N				

DECISION: CORYNEBACTERIUM DIPHTHERIAE MITIS 0.9241
 C. DIPHTHERIAE GRAVIS 0.8831
 C. DIPHTHERIAE INTERMEDIUS 0.3821

FCR CORYNEBACTERIUM DIPHTHERIAE MITIS	CHECK TOXIGENIC, HALO, AND UREA
WARNING: CORYNEBACTERIUM DIPHTHERIAE MITIS	IS EXTREMELY SERIOUS
FCR C. DIPHTHERIAE GRAVIS	CHECK TOXIGENIC, HALO, AND UREA
WARNING: C. DIPHTHERIAE GRAVIS	IS EXTREMELY SERIOUS
FCR C. DIPHTHERIAE INTERMEDIUS	CHECK TOXIGENIC, HALO, AND UREA
WARNING: C. DIPHTHERIAE INTERMEDIUS	IS EXTREMELY SERIOUS

Figure 5. After the technician enters the EKLM Test Results data, the screen displays the biochemical reactions, the three most probable organisms in probability order, and appropriate warnings.

EKLM TEST RESULTS

1 LACTOSE	N	2 DEXTROSE	P	3 DEXTROSE GAS	N
4 SUCROSE	N	5 MALTOSE	P	6 MANNITOL	P
7 XYLLOSE	P	8 GLYCEROL	N	9 MALONATE	N
10 UREA	N	11 MOTILITY	P	12 GELATIN (25 C)	N
13 INDOL	N	14 VP	N	15 S. CITRATE	N
16 AESCULIN HYD	N	17 H2S (KIA)	P	18 LYSINE	P
19 CRNITHINE	N	20 ARGinine	N	21 ACETATE	N
22 RHAMNOSE	N	23 ARABINOSE	N	24 DULCITOL	N
25 INOSITOL	N	26 ADONITOL	N	27 SALICIN	N
28 TREHALOSE	P	29 SORBITOL	P	30 RAFFINOSE	N
31 CNPG	N	32 J. TARTRATE	P	33 PHENYLALANINE	N
34 KCN	N	35 DNA (25 C)	N	36 YEL PIG (25 C)	N
37 MELIBIOSE	P	38 CELLOBIOSE	N	39 ARABITOL	N
40 METHYL RED	P	41 SEROTYPE	I		

DECISION: S. TYPHI 0.9860
 S. GALLINARUM 0.8538
 S. CHOLERAESUIS 0.8508

S. GALLINARUM VIOLATES MOTILITY NEGATIVE

WOULD YOU LIKE TO CHECK OTHER TESTS AGAINST THE CANDIDATES? Y

WHAT PERCENT LEVEL DO YOU WANT TO CHECK THE CANDIDATES? 100

ENTER THE NUMBER OF CANDIDATES TO BE CHECKED (1,2, OR 3): 2
 UNKNOWN SHOWS POSITIVE FOR MOTILITY BUT CURRENT DATA SHOWS THAT
 S. GALLINARUM ONLY HAS A 0.0% CHANCE OF TESTING POSITIVE

UNKNOWN SHOWS POSITIVE FOR MELIBIOSE BUT CURRENT DATA SHOWS THAT
 S. GALLINARUM ONLY HAS A 0.0% CHANCE OF TESTING POSITIVE

DO YOU WISH TO ENTER ANOTHER UNKNOWN (Y/N) ?

Figure 6. After the technician enters the EKLM Test Results data, the screen displays the same type of data as Figure 5, but instead of a Warnings field, prompts the technician to continue.

APPENDIX

BIOCHEMICAL TESTS

TESTS LIST #1

1 Lactose
 2 Dextrose
 3 Dextrose gas
 4 Sucrose
 5 Maltose
 6 Mannitol
 7 Xylose
 8 Glycerol
 9 Malonate
 10 Urea
 11 Motility
 12 Gelatin (25°C)
 13 Indol
 14 VP
 15 S. Citrate
 16 Aesuclin Hyd.
 17 H2S (KIA)
 18 Lysine
 19 Ornithine
 20 Arginine
 21 Acetate
 22 Rhamnose
 23 Arabinose
 24 Dulcitol
 25 Inositol
 26 Adonitol
 27 Salicin
 28 Trehalose
 29 Sorbitol
 30 Raffinose
 31 ONPG
 32 J. Tartrate
 33 Phenylalanine
 34 KCN
 35 DNA (25°C)
 36 Yel Pig (25°C)
 37 Melibiose
 38 Cellulose
 39 Arabitol
 40 Methyl Red
 41 Serotype

TESTS LIST #2

1 Fermentative
 2 Oxidase
 3 Dextrose Gas
 4 Lactose
 5 Dextrose
 6 Sucrose
 7 Maltose
 8 Mannitol
 9 Xylose
 10 Glycerol
 11 Rhamnose
 12 Arabinose
 13 Cellulose
 14 Salicin
 15 Trehalose
 16 Arabitol
 17 Urea
 18 Motility
 19 Gelatin
 20 Indol
 21 Nitrate
 22 VP
 23 S. Citrate
 24 Catalase
 25 Esculin
 26 H2S(KIA)
 27 KCN
 28 Lysine
 29 Ornithine
 30 Arginine
 31 Tyrosine
 32 J. Tartrate
 33 Acetate
 34 Mucate
 35 D'Nase
 36 Phenylalanine
 37 ONPG
 38 Lipase
 39 6% NaCl, growth
 40 10% NaCl, growth

TESTS LIST #3

1 Oxidative
 2 Fermentative
 3 Dextrose Gas
 4 MacConkey
 5 Lactose
 6 Dextrose
 7 Sucrose
 8 Maltose
 9 Mannitol
 10 Xylose
 11 Glycerol
 12 Fructose
 13 Urea
 14 Motility
 15 Gelatin
 16 Indol
 17 Nitrate
 18 VP
 19 S. Citrate
 20 Nut. Agar
 21 Oxidase
 22 Litmus Milk
 23 Catalase
 24 Esculin
 25 H2S Paper
 26 Growth (25°C)
 27 Growth (35°C)
 28 Growth (42°C)
 29 Lysine
 30 Ornithine
 31 Arginine
 32 Acetamide
 33 Tartrate
 34 Acetate
 35 Mucate
 36 D'Nase
 37 Pseudo agar
 38 Requires CO2
 39 Res. to Cephalothin
 40 Res. to Nalidixic Acid

TESTS LIST #4

- 1 MacConkey
- 2 SS
- 3 Dextrose
- 4 Lactose
- 5 Sucrose
- 6 Maltose
- 7 Mannitol
- 8 Xylose
- 9 Glycerol
- 10 Fructose
- 11 Urea
- 12 Motility
- 13 Gelatin
- 14 Indol
- 15 Nitrate
- 16 Gas from NO₃
- 17 Nitrite
- 18 S. Citrate
- 19 Nut. agar
- 20 Oxidase
- 21 Catalase
- 22 Litmus milk
- 23 Pseudosel
- 24 Esculin
- 25 H₂S, Butt
- 26 H₂S, paper
- 27 Growth, (25°C)
- 28 Growth, (35°C)
- 29 Growth, (42°C)
- 30 Lysine
- 31 Arginine
- 32 Ornithine
- 33 Tyrosine
- 34 Acetamide
- 35 Tartrate
- 36 Acetate
- 37 Mucate
- 38 D'Nase
- 39 Pseudo agar
- 40 Pigment
- 41 VP

TESTS LIST #5

- 1 Ala (porphyrin test)
- 2 V factor
- 3 Indol
- 4 Urea
- 5 Ornithine
- 6 hemolysis
- 7 Catalase
- 8 Oxidase
- 9 Lactose
- 10 Dextrose
- 11 Sucrose
- 12 Mannitol
- 13 Xylose
- 14 Trehalose
- 15 Nitrate
- 16 CO₂ enhanced growth
- 17 Serotype
- 18 Beta lactamase

TESTS LIST #6

- 1 Fermentative
- 2 MacConkey
- 3 Lactose
- 4 Dextrose
- 5 Sucrose
- 6 Maltose
- 7 Mannitol
- 8 Xylose
- 9 Glycerol
- 10 Fructose
- 11 Esculin
- 12 Urea
- 13 Motility
- 14 Gelatin
- 15 Indol
- 16 Nitrate
- 17 VP
- 18 S. Citrate
- 19 Nut. agar
- 20 Oxidase
- 21 Litmus milk
- 22 Catalase
- 23 Pseudosel
- 24 H₂S Paper
- 25 Growth (25°C)
- 26 Growth (35°C)
- 27 Growth (42°C)
- 28 Lysine
- 29 Ornithine
- 30 Arginine
- 31 Acetamide
- 32 J. Tartrate
- 33 Acetate
- 34 Mucate
- 35 D'Nase

TESTS LIST #7

1 VP
 2 Hippurate
 3 Esculin
 4 Pyrrolidonyl-
 arylamidase
 5 Alpha-Galactosidase
 6 Bet-Glucuronidase
 7 Beta-Galactosidase
 8 Alkaline phosphatase
 9 Leucine Arylamidase
 10 Arginine
 11 Ribase
 12 Arabinose
 13 Mannitol
 14 Sorbitol
 15 Lactose
 16 Trehalose
 17 Unulin
 18 Raffinose
 19 Starch
 20 Glycogen
 21 Beta hemolysis
 22 Dextran
 23 Levan

TESTS LIST #8

1 Oxidative
 2 Fermentative
 3 Lactose
 4 Dextrose
 5 Sucrose
 6 Maltose
 7 Mannitol
 8 Xylose
 9 Glycerol
 10 Fructose
 11 Urea
 12 Motility
 13 Gelatin
 14 Indol
 15 Nitrate
 16 S. Citrate
 17 Nutrient Agar
 18 Broth +6% NaCl
 19 Oxidase
 20 Litmus milk
 21 Catalase
 22 Aesculin
 23 H₂S, paper
 24 Growth, 25°C
 25 Growth, 35°C
 26 Growth, 42°C
 27 D'Nase
 28 Pigment
 29 Tinsdale, halo
 30 Toxigenic
 31 VP

TESTS LIST #9

1 Oxidative
 2 Fermentative
 3 Dextrose, Gas
 4 MacConkey
 5 Lactose
 6 Dextrose
 7 Sucrose
 8 Maltose
 9 Mannitol
 10 Xylose
 11 Glycerol
 12 Fructose
 13 Urea
 14 Gelatin
 15 Indol
 16 Nitrate
 17 Nitrite
 18 VP
 19 S. Citrate
 20 Nutrient Agar
 21 Oxidase
 22 Litmus milk
 23 Catalase
 24 Acetate
 25 H₂S, Paper
 26 Growth, 25°C
 27 Growth, 35°C
 28 Growth, 42°C
 29 Tyrosine
 30 Tartrate
 31 Mucate
 32 D'Nase
 33 Amylosucrase
 34 Phenylalanine
 35 ONPG
 36 Serotype

TESTS LIST #10

- 1 Indol
- 2 p-nitrophenyl- β -D-acetyl- β -D-glucosaminide
- 3 p-nitrophenyl- α -D-glucoside
- 4 p-nitrophenyl- α -L-arabinofuranoside
- 5 p-nitrophenyl- β -D-glucoside
- 6 p-nitrophenyl- α -L-fucoside
- 7 p-nitrophenyl-phosphate
- 8 p-nitrophenyl- α -D-galactoside
- 9 O-nitrophenyl B-D galactoside
- 10 Indoxyl-acetate
- 11 Arginine
- 12 L-Leucyl-4-methoxy- β -naphthylamide
- 13 L-proline- β -naphthylamide
- 14 L-pyrrolidonyl- β -naphthylamide
- 15 L-Tyrosine- β -naphthylamide
- 16 L-arginine- β -naphthylamine
- 17 L-alanyl-L-salanyl- β -naphthylamide
- 18 L-histidine- β -naphthylamide
- 19 L-phenylalanine- β -naphthylamide
- 20 L-Glycine- β -naphthylamide
- 21 Catalase

TESTS LIST #11

- 1 Colony, large
- 2 Pigment
- 3 Anaerobic
- 4 Aerobic
- 5 Coag.
- 6 Hemolysis
- 7 Nitrate
- 8 VP
- 9 Phosphate
- 10 Urease
- 11 Arg
- 12 Beta-Glucosidase
- 13 Beta-Glucoronide
- 14 Beta-Galactosidase
- 15 Novobiocin Res.
- 16 Trehalose
- 17 Mannitol
- 18 Xylose
- 19 Cellobiose
- 20 Sucrose
- 21 Mannose
- 22 Ribose
- 23 Raffinose
- 24 Lactose

TESTS LIST #12

- 1 Fermentative
- 2 Lactose
- 3 Dextrose
- 4 Sucrose
- 5 Maltose
- 6 Mannitol
- 7 Xylose
- 8 Glycerol
- 9 Fructose
- 10 Rhamnose
- 11 Urea
- 12 Motility
- 13 Gelatin
- 14 Indol
- 15 Nitrate
- 16 Nitrite
- 17 VP
- 18 S. Citrate
- 19 Nut. Agar
- 20 Broth + 6.5 NaCl
- 21 Oxidase
- 22 Litmus milk
- 23 Catalase
- 24 S. aureus Camp
- 25 Aesculin
- 26 H₂S Butt
- 27 H₂S paper
- 28 Growth 25°C
- 29 Growth 35°C
- 30 Growth 42°C
- 31 D'Nase
- 32 Beta hemolysis

TESTS LIST #13

1 Alpha hemolysis
 2 Gas, Dextrose, Thio
 3 Anaerobic growth
 4 Lactose
 5 Dextrose
 6 Sucrose
 7 Maltose
 8 Mannitol
 9 Xylose
 10 Fructose
 11 Rhamnose
 12 Arabinose
 13 Salicin
 14 Urea
 15 Motility
 16 Indol
 17 Nitrate
 18 S. Citrate
 19 Nut. Agar
 20 Oxidase
 21 Litmus milk, acid
 22 Catalase
 23 Aesculin
 24 KIA, A, slant
 25 H2S Paper
 26 Growth 25°C
 27 Growth 35°C
 28 Growth 42°C
 29 Prefer CO2

TESTS LIST # 14

1 Oxidative
 2 Fermentative
 3 Gas, Dextrose
 4 Lactose
 5 Dextrose
 6 Sucrose
 7 Maltose
 8 Mannitol
 9 Xylose
 10 Glycerol
 11 Fructose
 12 Salicin
 13 Urea
 14 Motility
 15 Gelatin
 16 Indol
 17 Nitrate
 18 VP
 19 S. Citrate
 20 Nut. Broth
 21 Nut. Broth +6% NaCl
 22 Oxidase
 23 Litmus Milk
 24 Catalase
 25 Aesculin
 26 KIA, But, acid
 27 KIAQ, Slant acid
 28 H2S, Butt
 29 H2S, Paper
 30 Growth, 25°C
 31 Growth, 35°C
 32 Growth, 42°C
 33 Lysine
 34 Ornithine
 35 Arginine
 36 D'Nase
 37 Acetamide
 38 Tartrate
 39 Acetate
 40 Mucate
 41 Starch
 42 Lecithinase
 43 Meth. Blue Red
 44 Hemolysis

TESTS LIST #15

1 Fermentative
 2 Gas, Dextrose
 3 MacConkey
 4 SS
 5 Lactose
 6 Dextrose
 7 Sucrose
 8 Maltose
 9 Mannitol
 10 Xylose
 11 Glycerol
 12 Fructose
 13 Urea
 14 Motility
 15 Gelatin
 16 Indol
 17 Nitrate
 18 Nitrite
 19 VP
 20 S. Citrate
 21 Nut. Agar
 22 Oxidase
 23 Litmus Milk
 24 Catalase
 25 Pseudosel
 26 Aesculin
 27 KIA, Butt, acid
 28 KIA, Slant, acid
 29 H2S, Butt
 30 H2S, Paper
 31 Growth 25°C
 32 Growth 35°C
 33 Growth 42°C
 34 Lysine
 35 Ornithine
 36 Arginine
 37 Tyrosine
 38 Acetamide
 39 Tartrate
 40 Acetate
 41 Mucate
 42 D'Nase
 43 Amylosucrose
 44 Hemolysis
 45 Pigment